PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

# PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>12 February 2001 (12.02.01) | |
| **International application No.**<br>PCT/GB00/02100 | **Applicant's or agent's file reference**<br>P.6195 WOP |
| **International filing date** (day/month/year)<br>09 June 2000 (09.06.00) | **Priority date** (day/month/year)<br>11 June 1999 (11.06.99) |
| **Applicant**<br>PIRZAD, Ramin | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

03 January 2001 (03.01.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland** | Authorized officer<br>S. Mafla |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

Form PCT/IB/331 (July 1992)

GB0002100

# PATENT COOPERATION TREATY

## PCT

From the INTERNATIONAL BUREAU

To:

MAGUIRE BOSS
5 Crown Street
St. Ives
Cambridgeshire PE27 5EB
ROYAUME-UNI

## NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>23 October 2001 (23.10.01) | |
| **Applicant's or agent's file reference**<br>P.6195 WOP | **IMPORTANT NOTIFICATION** |
| **International application No.**<br>PCT/GB00/02100 | **International filing date** (day/month/year)<br>09 June 2000 (09.06.00) |

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

| Name and Address | State of Nationality | State of Residence |
|---|---|---|
| PIRZAD, Ramin<br>40 Nursery Gardens<br>St. Ives<br>Cambridgeshire PE21 3NL<br>United Kingdom | GB | GB |
| | Telephone No. | |
| | Facsimile No. | |
| | Teleprinter No. | |

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

| Name and Address | State of Nationality | State of Residence |
|---|---|---|
| ACARIS HEALTHCARE SOLUTIONS PLC<br>Daedalus House<br>Station Road<br>Cambridge CB1 2RE<br>United Kingdom | GB | GB |
| | Telephone No. | |
| | Facsimile No. | |
| | Teleprinter No. | |

3. Further observations, if necessary:
**Due to assignment of rights, the person in Box 2 has been recorded as applicant for all designated States except US, the person in Box 1 remains inventor and applicant for US only.**

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[ ] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

| | |
|---|---|
| The International Bureau f WIPO<br>34, chemin des Colombettes<br>1211 G neva 20, Switz rland | Authorized officer<br>Anman QIU |
| Facsimile No.: (41-22) 740.14.35 | Teleph ne No.: (41-22) 338.83.38 |

Form PCT/IB/306 (March 1994)

004393223

# PATENT COOPERATION TREATY

# PCT

REC'D 27 SEP 2001
WIPO PCT

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference<br>P.6195 WOP | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| International application No.<br>PCT/GB00/02100 | International filing date *(day/month/year)*<br>09/06/2000 | Priority date *(day/month/year)*<br>11/06/1999 |
| International Patent Classification (IPC) or national classification and IPC<br>G01N31/22 | | |
| Applicant<br>PIRZAD, Ramin | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 03/01/2001 | 25.09.2001 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Klee, B<br>Telephone No. +49 89 2399 2675 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/02100

## I. Basis of the r port

1. With regard to the **elem nts** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
**Description, pages:**

| | | | | | |
|---|---|---|---|---|---|
| 1-15 | as originally filed | | | | |

**Claims, No.:**

| | | | | | |
|---|---|---|---|---|---|
| 1-30 | as received on | 11/09/2001 | with letter of | 05/09/2001 | |

**Drawings, sheets:**

| | | | | | |
|---|---|---|---|---|---|
| 1/4-4/4 | as originally filed | | | | |

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-28, 30 |
| | No: | Claims | 29 |
| Inventive step (IS) | Yes: | Claims | 1-28, |
| | No: | Claims | 30 |
| Industrial applicability (IA) | Yes: | Claims | 1-30 |
| | No: | Claims | |

2. Citations and explanations
**see separate sheet**

**R f r nc s cited:**

D1: WO 99 10736 A (WHITE STEPHEN ;UNIV CRANFIELD (GB); TURNER ANTHONY PETER FRANCIS () 4 March 1999 (1999-03-04)

D2: DATABASE WPI Section Ch, Week 198537 Derwent Publications Ltd., London, GB; Class A96, AN 1985-226925 XP002150017 & JP 60 147651 A (SEKISUI CHEM IND CO LTD) , 3 August 1985 (1985-08-03)

D3: WO 96 30764 A (VORWERK CO INTERHOLDING ;POCH HEIKE (DE); SAUER RALF (DE); SINCLAI) 3 October 1996 (1996-10-03)

D4: CAYOT P., TAINTURIER G.: 'The Quantification of Protein Amino Groups by the Trinitrobenzenesulfonic Acid method: A Reexamination' ANALYTICAL BIOCHEMISTRY, vol. 249, 1997, pages 184-200, XP002150016

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step r industrial applicability; citations and explanations supporting such statement**

1. Novelty (Art.33(2) PCT) and Inventive step Art.33 (3) PCT

1.1 With respect to claim 1

Document D1 is regarded as being the closest prior art to the subject-matter of claim 1, and shows a method of detecting the level of protein in dust (page 1, lines 3-6), comprising:

providing a dust sample (page 5, lines 19, 20);

liberating from the dust sample at least one component selected from the group consisting of aliphatic amines and aliphatic amino acids (page 1, line 8, "liberating amino acids); determining the relative concentration of the liberated at least one component (page 1, line 9-11 ".. detection of amino acids"); and providing an indication of allergen activity (page 1, line 19) in dependence upon relative concentration determined (page 1, lines 3-25).

The subject-matter of claim 1 therefore differs from this known in D1 in that breakdown components of proteins or peptides are extracted without subjecting to degradation by proteolytic enzyme and liberation of amino acids and that the extracted at least one breakdown component is reacted with a colorimetric amine detection reagent and the intensity is quantitatively measured of any resulting coloration, the allergen activity being proportional to the intensity of coloration.

The subject-matter of claim 1 is therefore novel (Article 33(2) PCT).

The problem to be solved by the present invention may therefore be regarded as to provide a method to determine allergen activity of a dust sample due to the dust mite activity.
None of the documents D1-D4 describes the **extraction** of **breakdown components of proteins or peptides** and their detection by a colorimetric method. Nor does any of the documents cited indicate that the mere detection of breakdown components and peptides already liberated by proteases contained in the sample excreted from mites could be used as an indicator for allergen activity due to mite activity. In contrast D1 and D3 determine the total protein content of a dust sample not distinguishing from the already existing breakdown components. None of the documents cited gives a hint that the breakdown components are an indicator for allergen activity in dust samples, due to the activity of the mite originating proteases. Therefore claim 1 is inventive.

1.2 With respect to claim 16
The same as discussed under item 1.1 applies to independent claim 16. Moreover none of the references cited describes a method of determining allergen activity in dust comprising the step of providing a protease substrate having immobilized thereon proteins or peptides labelled with a chromogenic substance and quantitatively measuring the breakdown components as claimed in claim 16.

1.3 Claims 2-15 and 17-20 are dependent on claims 1 or 16 respectively and as such also meet the requirements of the PCT with respect to novelty and inventive step.

1.4 With respect to claim 21
None of the references cited describes or gives an indication to set up such a kit apparatus comprising
- a first chamber comprising a surfactant
- a second chamber comprising a colorimetric amine detection reagent
- means for quantitatively measuring the intensity of any coloration resulting from reacting the extract-containing surfactant and the colorimetric amine detection reagent;
- and means for indicating relative level of allergen activity in the dust sample based on the quantitative measurement. Therefore claim 21 is new and inventive

(see also item 1.1).

1.5 With respect to claim 29

The only feature which characterizes the apparatus of claim 29 is a substrate having immobilized thereon proteins or peptids labelled with a chromogenic substance. A person skilled in the art for example working in the field of biosensors uses proteins labelled with chromogenic substances to test the ability of surfaces to bind proteins, for example to test for nonspecific binding. Therefore surfaces having immobilized thereon proteins labelled with a chromogen are known to a person skilled in the art. Thus claim 29 is not new.

1.6 With respect to claim 30

Albumin is a protein which is inexpensive, commercially available and the azo-group is commonly used to covalently couple a further component to the protein or to immobilize the protein, therefore claim 30 does not contain any features which, in combination with the features of claim 29 to which it refers, meet the requirements of the PCT in respect of inventive step.

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file referenc | FOR FURTHER ACTION | see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. |
|---|---|---|
| P.6195 WOP | | |
| International application No. | International filing date *(day/month/year)* | (Earliest) Priority Date *(day/month/year)* |
| PCT/GB 00/ 02100 | 09/06/2000 | 11/06/1999 |
| Applicant | | |
| PIRZAD, Ramin | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in th language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in th international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. Th applicant may, within one month from the dat of mailing of this international search report, submit comments to this Authority.

6. The figure of th **drawings** to be published with the abstract is Figure No. 3

   [X] as suggested by the applicant.

   [ ] because th applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of th figures.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/02100

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N31/22 C12Q1/37 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA, INSPEC, COMPENDEX, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 99 10736 A (WHITE STEPHEN ;UNIV CRANFIELD (GB); TURNER ANTHONY PETER FRANCIS () 4 March 1999 (1999-03-04)<br>page 1, line 3 - line 25<br>page 4, line 13 -page 5, line 32<br>examples 1,4,6<br>--- | 1,2, 4-12,16, 18,19, 21-25, 27-30, 34,35 |
| Y | DATABASE WPI<br>Section Ch, Week 198537<br>Derwent Publications Ltd., London, GB;<br>Class A96, AN 1985-226925<br>XP002150017<br>& JP 60 147651 A (SEKISUI CHEM IND CO LTD)<br>, 3 August 1985 (1985-08-03)<br>abstract<br>---<br>-/-- | 1,2, 4-12,16, 18,19, 21-25, 27-30, 34,35 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 13 October 2000 | 27/10/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Menidjel, R |

Form PCT/ISA/210 (second sheet) (July 1992)

International Application No
PCT/GB 00/02100

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | WO 96 30764 A (VORWERK CO INTERHOLDING ;POCH HEIKE (DE); SAUER RALF (DE); SINCLAI) 3 October 1996 (1996-10-03)<br><br>abstract<br>page 1, line 1 - line 24<br>page 2, line 47 -page 3, line 88 | 1,6, 8-13, 21-23, 27-32,34 |
| Y | CAYOT P., TAINTURIER G.: "The Quantification of Protein Amino Groups by the Trinitrobenzenesulfonic Acid method: A Reexamination"<br>ANALYTICAL BIOCHEMISTRY,<br>vol. 249, 1997, pages 184-200, XP002150016<br>abstract<br>page 185, right-hand column, paragraph 2 | 1,6, 8-13, 21-23, 27-32,34 |

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/02100

| Patent document cited in search report | Publication date | Patent family member(s) | Publication dat |
|---|---|---|---|
| WO 9910736 A | 04-03-1999 | AU 8869998 A | 16-03-1999 |
| JP 60147651 A | 03-08-1985 | JP 1719344 C | 14-12-1992 |
| | | JP 4003504 B | 23-01-1992 |
| WO 9630764 A | 03-10-1996 | DE 19518287 A | 26-09-1996 |
| | | AU 5144696 A | 16-10-1996 |
| | | CZ 9702727 A | 18-02-1998 |
| | | EP 0815451 A | 07-01-1998 |
| | | JP 11502621 T | 02-03-1999 |
| | | PL 322449 A | 02-02-1998 |
| | | US 5981287 A | 09-11-1999 |

# PATENT COOPERATION TREATY



From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

MAGUIRE BOSS
5 Crown Street
St. Ives
Cambridgeshire PE27 5EB
GRANDE BRETAGNE

# PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

| | |
|---|---|
| Date of mailing *(day/month/year)* | 25.09.2001 |
| Applicant's or agent's file reference: P.6195 WOP | **IMPORTANT NOTIFICATION** |

| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
|---|---|---|
| PCT/GB00/02100 | 09/06/2000 | 11/06/1999 |

Applicant
PIRZAD, Ramin

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

| Name and mailing address of the IPEA/ | Authorized officer |
|---|---|
| European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Weber, R<br><br>Tel.+49 89 2399-2382 |

Form PCT/IPEA/416 (July 1992)

# PATENT COOPERATION TREATY

# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| P.6195 WOP | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/GB00/02100 | 09/06/2000 | 11/06/1999 |

International Patent Classification (IPC) or national classification and IPC
G01N31/22

Applicant
PIRZAD, Ramin

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which hav been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 03/01/2001 | 25.09.2001 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Offic<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Klee, B<br>Telephone No. +49 89 2399 2675 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB00/02100

## I. Basis of th r port

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
**Description, pages:**

1-15 as originally filed

**Claims, No.:**

1-30 as received on 11/09/2001 with letter of 05/09/2001

**Drawings, sheets:**

1/4-4/4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | |
|---|---|---|
| Novelty (N) | Yes: | Claims 1-28, 30 |
| | No: | Claims 29 |
| Inventive step (IS) | Yes: | Claims 1-28, |
| | No: | Claims 30 |
| Industrial applicability (IA) | Yes: | Claims 1-30 |
| | No: | Claims |

2. Citations and explanations
**see separate sheet**

**Refer nc s cit d:**

D1: WO 99 10736 A (WHITE STEPHEN ;UNIV CRANFIELD (GB); TURNER ANTHONY PETER FRANCIS () 4 March 1999 (1999-03-04)

D2: DATABASE WPI Section Ch, Week 198537 Derwent Publications Ltd., London, GB; Class A96, AN 1985-226925 XP002150017 & JP 60 147651 A (SEKISUI CHEM IND CO LTD) , 3 August 1985 (1985-08-03)

D3: WO 96 30764 A (VORWERK CO INTERHOLDING ;POCH HEIKE (DE); SAUER RALF (DE); SINCLAI) 3 October 1996 (1996-10-03)

D4: CAYOT P., TAINTURIER G.: 'The Quantification of Protein Amino Groups by the Trinitrobenzenesulfonic Acid method: A Reexamination' ANALYTICAL BIOCHEMISTRY, vol. 249, 1997, pages 184-200, XP002150016

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Novelty (Art.33(2) PCT) and Inventive step Art.33 (3) PCT

1.1 With respect to claim 1

Document D1 is regarded as being the closest prior art to the subject-matter of claim 1, and shows a method of detecting the level of protein in dust (page 1, lines 3-6), comprising:

providing a dust sample (page 5, lines 19, 20);

liberating from the dust sample at least one component selected from the group consisting of aliphatic amines and aliphatic amino acids (page 1, line 8, "liberating amino acids); determining the relative concentration of the liberated at least one component (page 1, line 9-11 ".. detection of amino acids"); and providing an indication of allergen activity (page 1, line 19) in dependence upon relative concentration determined (page 1, lines 3-25).

The subject-matter of claim 1 therefore differs from this known in D1 in that breakdown components of proteins or peptides are extracted without subjecting to degradation by proteolytic enzyme and liberation of amino acids and that the extracted at least one breakdown component is reacted with a colorimetric amine detection reagent and the intensity is quantitatively measured of any resulting coloration, the allergen activity being proportional to the intensity of coloration. The subject-matter of claim 1 is therefore novel (Article 33(2) PCT).

The problem to be solved by the present invention may therefore be regarded as to provide a method to determine allergen activity of a dust sample due to the dust mite activity.
None of the documents D1-D4 describes the **extraction** of **breakdown components of proteins or peptides** and their detection by a colorimetric method. Nor does any of the documents cited indicate that the mere detection of breakdown components and peptides already liberated by proteases contained in the sample excreted from mites could be used as an indicator for allergen activity due to mite activity. In contrast D1 and D3 determine the total protein content of a dust sample not distinguishing from the already existing breakdown components. None of the documents cited gives a hint that the breakdown components are an indicator for allergen activity in dust samples, due to the activity of the mite originating proteases. Therefore claim 1 is inventive.

1.2 With respect to claim 16
The same as discussed under item 1.1 applies to independent claim 16. Moreover none of the references cited describes a method of determining allergen activity in dust comprising the step of providing a protease substrate having immobilized thereon proteins or peptides labelled with a chromogenic substance and quantitatively measuring the breakdown components as claimed in claim 16.

1.3 Claims 2-15 and 17-20 are dependent on claims 1 or 16 respectively and as such also meet the requirements of the PCT with respect to novelty and inventive step.

1.4 With respect to claim 21
None of the references cited describes or gives an indication to set up such a kit apparatus comprising
- a first chamber comprising a surfactant
- a second chamber comprising a colorimetric amine detection reagent
- means for quantitatively measuring the intensity of any coloration resulting from reacting the extract-containing surfactant and the colorimetric amine detection reagent;
- and means for indicating relative level of allergen activity in the dust sample based on the quantitative measurement. Therefore claim 21 is new and inventive

(see also item 1.1).

1.5 With respect to claim 29

The only feature which characterizes the apparatus of claim 29 is a substrate having immobilized thereon proteins or peptids labelled with a chromogenic substance. A person skilled in the art for example working in the field of biosensors uses proteins labelled with chromogenic substances to test the ability of surfaces to bind proteins, for example to test for nonspecific binding. Therefore surfaces having immobilized thereon proteins labelled with a chromogen are known to a person skilled in the art. Thus claim 29 is not new.

1.6 With respect to claim 30

Albumin is a protein which is inexpensive, commercially available and the azo-group is commonly used to covalently couple a further component to the protein or to immobilize the protein, therefore claim 30 does not contain any features which, in combination with the features of claim 29 to which it refers, meet the requirements of the PCT in respect of inventive step.

CLAIMS

1. A method of determining allergen activity in dust, comprising:

providing a dust sample;

extracting from the dust sample at least one breakdown component of proteins or peptides;

reacting the extracted at least one breakdown component with a colorimetric amine detection reagent; and

quantitatively measuring the intensity of any resulting coloration, the allergen activity being proportional to the intensity of coloration.

2. A method according to claim 1, further comprising exposing the dust sample to a protease substrate, the protease substrate having immobilised thereon a protein or peptide on which protease in the dust sample may act.

3. A method according to claim 2, further comprising adding a protease inhibitor to the dust sample to suppress activity of a specific protease prior to exposure to the protease substrate.

4. A method according to claim 2, in which the protease substrate is protease specific, with only a specific protease being able to act on the protein or peptide immobilised on the substrate.

5. A method according to claim 2,3 or 4, in which the protease substrate comprises a filter to facilitate extraction of mobile breakdown components of the protein or peptide immobilised on the protease substrate.

6. A method according to any one of claims 1 to 5, in

which the breakdown components extracted from the dust sample include amines, amino acids or peptides present in the dust sample.

7. A method according to any one of claims 1 to 6, in which the colorimetric amine detection reagent is 2,4,6-trinitrobenzene sulphonic acid, (hereinafter referred to as TNBSA)

8. A method according to any one of claims 1 to 7, in which the at least one breakdown component is extracted by bringing the dust sample into contact with a surface active agent (surfactant).

9. A method according to claim 8, further comprising separating any dust sample solid residues from the surfactant prior to reacting with the colorimetric detection reagent.

10. A method according to claim 8 or 9, in which the surfactant is an aqueous solution comprising sodium dodecyl sulphate.

11. A method according to claim 10, in which the aqueous solution is alkaline.

12. A method according to claim 10 or 11, in which the aqueous solution further comprises sodium hydrogen carbonate.

13. A method according to any one of claims 1 to 12, in which the intensity of any resulting coloration is quantitatively measured by comparison with at least one reference colour.

14. A method according to claim 13, in which different

colour references are selected to indicate at least three different kinds of allergen activity.

15. A method according to any one of claims 1 to 14, further comprising preserving the reaction mixture by using a stopping agent after a pre-selected incubation period.

16. A method of determining allergen activity in dust, comprising:

providing a dust sample;

providing a protease substrate, the protease substrate having immobilised thereon proteins or peptides labelled with a chromogenic substance;

exposing the protease substrate to the dust sample under conditions whereby a protease in the dust sample may act on the immobilised protein or peptide to produce mobile breakdown components labelled with the chromogenic substance;

and quantitatively measuring the intensity of any resulting coloration, the allergen activity being proportional to the intensity of the coloration.

17. A method according to claim 16, further comprising adding a protease inhibitor to the dust sample to suppress activity of a specific protease prior to exposure to the protease substrate.

18. A method according to claim 16, in which the protease substrate is protease specific, with only a specific protease being able to act on the proteins or peptides immobilised on the substrate.

19. A method according to claim 16,17 or 18, in which the protease substrate comprises a filter to facilitate extraction of mobile breakdown components labelled with the chromogenic substance.

20. A method according to any one of claims 16 to 19, in which the intensity of any resulting coloration is quantitatively determined by comparison with at least one reference colour.

21. Kit apparatus for use in a domestic environment for indicating allergen levels in dust, comprising a first chamber comprising a surfactant for extracting from a dust sample at least one breakdown component of proteins and peptides; a second chamber comprising a colorimetric amine detection reagent; means for quantitatively measuring the intensity of any coloration resulting from reacting the extract-containing surfactant and the colorimetric amine detection reagent; and means for indicating relative level of allergen activity in the dust sample based on the quantitative measurement.

22. Kit apparatus according to claim 21, further comprising a filter for filtering dust sample solid residues from the surfactant before reacting with the colorimetric amine detection reagent.

23. Kit apparatus according to claim 21 or 22, in which one of the two chambers has the capacity to receive the contents of the other chamber.

24. Kit apparatus according to claim 23, in which the second chamber has the capacity to hold the colorimetric

amine detection reagent and the surfactant.

25. Kit apparatus according to any one of claims 21 to 24, in which the quantitative measuring means comprises at least one colour reference, against which the intensity of any coloration may be compared.

26. Kit apparatus according to any one of claims 21 to 24, in which the indicating means comprises a scale, which is linked to the intensity of any coloration measured.

27. Kit apparatus according to any one of claims 21 to 24, further comprising a third chamber comprising a stopping reagent to limit the reaction between the extract-containing surfactant and the colorimetric amine detection reagent.

28. Kit apparatus according to any one of claims 21 to 27, in which the colorimetric amine detection reagent is 2,4,6-trinitrobenzene sulphonic acid.

29. Apparatus for use in determining allergen levels in a dust sample, comprising a protease substrate having immobilised thereon proteins or peptides labelled with a chromogenic substance, whereby any protease in the dust sample may act on the immobilised proteins or peptides to produce mobile breakdown components labelled with the chromogenic substance.

30. Apparatus according to claim 20, in which proteins labelled with chromogenic the substance comprise azo-albumin.